Pediatr Nephrol (1991) 5: 461–466

Pediatric Nephrology

Growth

Original article

Effect of vitamin D on growth cartilage cell proliferation in vitro

G. Klaus[1], R. Meinhold-Heerlein[1], P. Milde[2], E. Ritz[2], and O. Mehls[1]

Division of Nephrology, Departments of [1] Paediatrics and [2] Internal Medicine, University of Heidelberg, W-6900 Heidelberg, Federal Republic of Germany

Received November 26, 1990; accepted December 13, 1990

Abstract. Disturbed calcification of the growth plate and stunting is a frequent finding in vitamin D-deficiency rickets, vitamin D-dependency rickets and renal osteodystrophy, illustrating that chondrocytes are a target for vitamin D. This observation prompted an investigation of Ic, 25-dihydroxy vitamin D_3 [1,25$(OH)_2D_3$] receptor expression and action of vitamin D metabolites on chondrocyte proliferation. In tibial growth plates and in primary cultures of tibial growth cartilage of male Sprague-Dawley rats (80 g) specific binding of [^{3}H]-1,25$(OH)_2D_3$ was noted. Scatchard analysis revealed the presence of a single class of non-interacting binding sites. K_d was 10^{-11} M irrespective of growth phase. The binding macromolecule had a sedimentation coefficient of 3.5 S. Interaction with DNA was demonstrated by DNA-cellulose affinity chromatography. By immunohistology, growth cartilage cells (rabbit tibia) were shown to express nuclear 1,25$(OH)_2D_3$ receptors, most prominently in the proliferative and early hypertrophic zone. This corresponds to binding data which showed highest binding of 1,25$(OH)_2D_3$ in the logarithmic growth phase (12,780 molecules/cell versus 4,538 molecules/cell in confluent cells) in primary cultures of growth plate chondrocytes. In the presence of delipidated fetal calf serum 1,25$(OH)_2D_3$ had a biphasic effect on cell proliferation and density, i.e. stimulation at 10^{-12} M and dose-dependent inhibition at 10^{-10} M and below. Inhibition was specific and not seen with 24 (R), 25-dihydroxyvitamin D_3 or dexamethasone. Growth phase-dependent 1,25$(OH)_2D_3$ receptor expression and specific effects of 1,25$(OH)_2D_3$ on chrondrocyte proliferation in vitro point to a role for vitamin D in the homeostasis of growth cartilage of the rat.

Key words: Growth cartilage – Vitamin D – Rickets – Chondrocytes

Offprint requests to: G. Klaus, Division of Nephrology, Department of Paediatrics, University of Heidelberg, Im Neuenheimer Feld 150, W-6900 Heidelberg, Federal Republic of Germany

Introduction

Disturbance of the growth plate and stunting is a hallmark of vitamin D-deficiency rickets, vitamin D-dependency rickets and renal osteodystrophy. These conditions are characterized by a decrease in mean circulating Iα, 25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] levels, although individual levels may be in the normal range [1, 2]. Administration of vitamin D rapidly corrects this disorder and reverses stunting in rickets, but not in renal osteodystrophy. This suggests that growth cartilage is a target organ for vitamin D. It has remained unclear, however, to what extent the positive effect of vitamin D on growth is mediated by direct action of vitamin D metabolites on chondrocytes or by indirect systemic effects such as altered concentrations of electrolytes and calciotropic hormones (calcium, phosphorus, parathyroid hormone); direct effects of vitamin D would necessitate interaction of active vitamin D metabolites with chondrocytes of the growth plate as target cells.

Evidence for a direct action of vitamin D on growth plate chondrocytes has been reported by many authors [3–7]. Suda et al. [3] demonstrated vitamin D receptors in embryonal chicken chondrocytes in vitro. Takigawa et al. [4] demonstrated that 1,25$(OH)_2D_3$ stimulated growth in rapidly proliferating, but poorly differentiated costal chondrocytes of rabbits and suppressed the expression of the cartilage phenotype. Silbermann et al. [5, 8] reported an inhibitory effect of 1,25$(OH)_2D_3$ on chondrocyte proliferation in vivo. Furthermore Soemjen et al. [9] published evidence for a specific role of 24(R), 25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] in the growth and differentiation of developing cartilage cells. In these studies the action of 24,25$(OH)_2D_3$ was not duplicated by 1,25$(OH)_2D_3$. In view of these conflicting results we analysed: (1) 1,25$(OH)_2D_3$ receptor expression and regulation and (2) actions of vitamin D metabolites on proliferation of growth plate chondrocytes of rats in vitro.

Materials and methods

1,25(OH)$_2$[26,27-methyl-^{3}H]cholecalciferol (158 Ci/mmol) and 25(OH)[26,27-methyl-^{3}H]cholecalciferol were obtained from Amersham Buchler (Braunschweig, FRG); unlabelled 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$ were gifts from Dr. Calcanis (Hoffmann-La Roche, Grenzach, FRG); 24,25(OH)$_2$D$_3$ was purchased from Duphar (Amsterdam, The Netherlands); [^{3}H]thymidine (40 Ci/mmol), [^{14}C]-methylated ovalbumin (20 μCi/mmol) and [^{14}C]-methylated globulin (25 μCi/mmol) were obtained from New England Nuclear (Dreieich, FRG); hydroxylapatite, dithiothreitol and Triton X-100 were purchased from Sigma (Munich, FRG); F-12 medium, phosphate-buffered saline, gentamicin, *Clostridium* collagenase (EC 3.4.24.3), DNase I (EC 3.1.21.1) and trypan blue were obtained from Boehringer (Mannheim, FRG). The monoclonal antibody (mAb) 9A7τ2b was a gift from Dr. Haussler (University of Arizona, Arizona, USA).

Male Sprague-Dawley rats (80 g) were killed by cervical dislocation under deep ether anaesthesia; after soaking the distal part of the body with 70% ethanol, both tibiae were dissected free.

Epiphyseal chondrocytes were isolated and cultured as described by Benya and Shaffer [10] and Lindahl et al. [11]. In brief, the epiphyseal growth plates of proximal tibia, cleaned of perichondrium, were dissected microscopically and digested for 3 h at 37° C by clostridial collagenase (0.12% w/v) and 0.02% w/v bacterial DNase in F-12 medium. Dissociated cells were counted using a Neubauer chamber; viability was determined by the trypan blue exclusion technique and always exceeded 90%.

Cells were cultured in F-12 medium supplemented with L-ascorbic acid (50 μg/ml), HEPES (10 mmol/l), gentamicin (100 μg/ml) and 10% fetal calf serum (FCS) or 10% charcoal-treated FCS (delipidated FCS) at 37° C in 5% CO_2. No vitamin D metabolites were demonstrable in charcoal-treated FCS. Vitamin D metabolites were dissolved in ethanol (0.05% final concentration) and were added with fresh medium every 2nd day. Cells were cultured in monolayers or in agarose-stabilized suspension cultures. Monolayers were produced by plating at 1×10^5 cells/ml in 35-mm or 100-mm plastic dishes (Falcon, Oxnard, Calif.) and were used for 1,25(OH)$_2$D$_3$ receptor studies and proliferation assays.

In a second set of experiments cells were cultured in agarose according to Benya and Shaffer [10]. Petri dishes (60 mm) were coated with a bottom film of 1% standard low agarose (Bio-Rad, richmond, Calif.) in water. Low gel temperature (LGT) agarose (Bio-Rad) was mixed with an equal volume of double-concentrated F-12 medium to give a final concentration of 1% agarose. The cell suspension (F-12 medium with 10% delipidated FCS, 160,000 cells/ml) was then mixed with 1% LGT agarose to give a final cell concentration of 80,000 cells/ml. Two millilitres of the LGT cell suspension were added to the dishes, which were kept at 37° C for 10 min before gelation at 4° C (10 min). Subsequently, 3 ml F-12 medium supplemented with 10% delipidated FCS and various concentrations of vitamin D metabolites (or solvent control) were added on top of th solidified agarose. The cultures were screened for adherent cell clusters of more than three cells. No such clusters were seen at the start of culture in any experiments presented in this study.

Proliferation assays

Cell counts. Cells in monolayer cultures were counted in the logarithmic growth phase and at confluence using a Neubauer chamber.

Clonal assay. Suspension cultures were terminated by fixation in buffered formaldehyde (4%) on day 21. A colony was defined as a cluster of cells with matrix stained by alcian blue. Colonies were counted in 100 squares (2-mm grid) for each dish.

1,25(OH)$_2$D$_3$ receptor assay. Cell extracts in KTED buffer (0.4 M KCl, 10 mM Tris HCl, 1.5 mM EDTA, 2 mM dithiothreitol, 10 mM sodium molybdate, pH 7.4) were prepared and purified as described elsewhere [12].

Sucrose density gradient. Sucrose density gradient analysis was carried out as described [13]. In brief, KTED cell extracts were incubated with [^{3}H]-1,25(OH)$_2$D$_3$ alone or with a 100-fold molar excess of unlabelled 1,25(OH)$_2$D$_3$ or other vitamin D metabolites, and layered on top of equilibrated 5%–20% sucrose density gradients and centrifuged at 255,000 *g* for 21 h at 4° C (SW 60 rotor, Beckman Instruments, Fullerton CA). Seven-drop fractions were collected and labelled with [^{3}C]-1,25(OH)$_2$D$_3$ alone or a 100-fold molar excess of 1,25(OH)$_2$D$_3$. Bound [^{3}H]-1,25(OH)$_2$D$_3$ was quantitated by hydroxylapatite assay [14].

Scatchard analysis. Saturation analysis according to Scatchard [15] was carried out as described [12]. Aliquots of cell KTED extracts (100 μl, protein concentration 0.5–1.2 mg/ml) were incubated for 16 h at 4° C with increasing concentrations (0.1–5.0 nM) of [^{3}H]-1,25(OH)$_2$D$_3$ in the absence or presence of 100-fold molar excess of 1,25(OH)$_2$D$_3$. Bound [^{3}H]-1,25(OH)$_2$D$_3$ was determined using the hydroxylapatite assay [14].

Statistical analysis. Data are given as mean ± SD. Statistical significance was analysed using the Mann-Whitney U test.

Results

Regulation of the 1,25(OH)$_2$D$_3$ receptor in chondrocytes

Specific binding of 1,25(OH)$_2$D$_3$ was examined in the tibial growth plate and in cultured growth plate chondrocytes of rats. Specific binding of [^{3}H]-1,25(OH)$_2$D$_3$ by a macromolecule sedimenting at 3.5 S in homogenized growth plates is shown in Fig. 1. The sedimentation coefficient corresponds to that of mammalian 1,25(OH)$_2$D$_3$ receptors [12, 16]. Radioactive hormone was displaced by 100-fold molar excess of unlabelled 1,25(OH)$_2$D$_3$. Similar results were obtained from cultured chondrocytes of the growth plate, but not xiphoid cartilage.

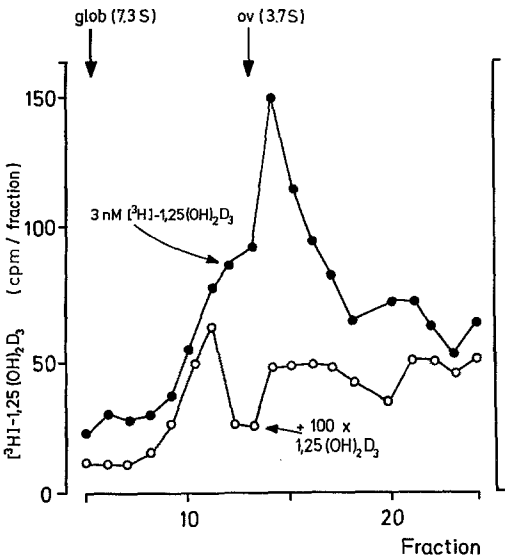

Fig. 1. Sucrose density gradient analysis of KTED extracts of homogenized tibial growth plates. The graph shows binding of [^{3}H]-1α, 25-dihydroxyvitamin D$_3$ ([^{3}H]-1,25(OH)$_2$D$_3$ by a 3.5-S macromolecule. Binding is reversed by a 100-fold molar excess of unlabelled 1,25(OH)$_2$D$_3$. Bovine gammaglobulin (*glob*) and ovalbumin (*ov*) were used as markers at 7.3 S and 3.7 S, respectively

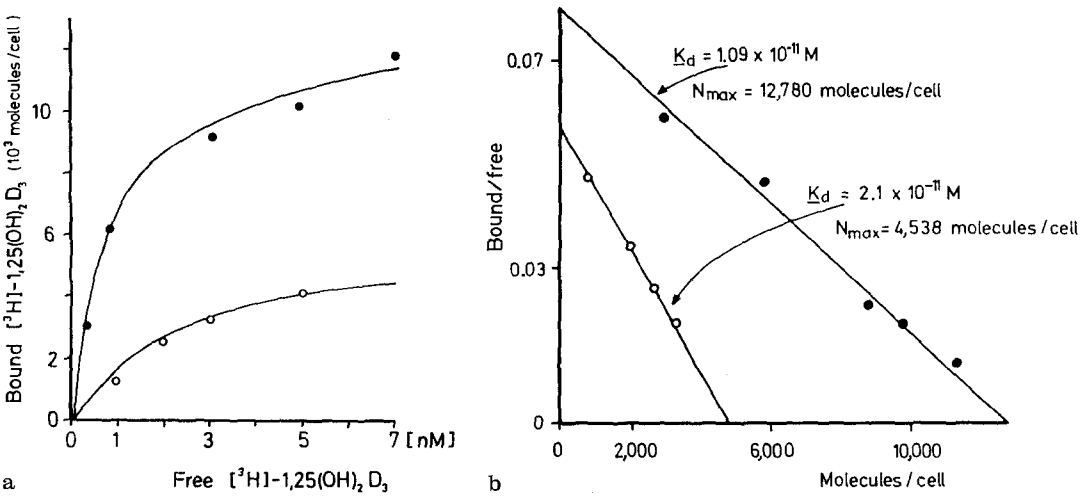

Fig. 2. Regulation of $1,25(OH)_2D_3$ receptor expression in chondrocytes *in vitro*. Growing (● 7×10^4 cells/cm^2) and confluent (○, 12×10^4 cells/cm^2) cells were analysed by saturation analysis. **a** The saturation curve shows specific binding of [^{3}H]-$1,25(OH)_2D_3$ with a plateau at 5 nM for all groups. **b** The equilibrium dissociation constants K_d were calculated from the slope of the regression lines, as depicted in the Scatchard plots. Maximum binding (N_{max}) was determined by extrapolation of the lines to the abscissa

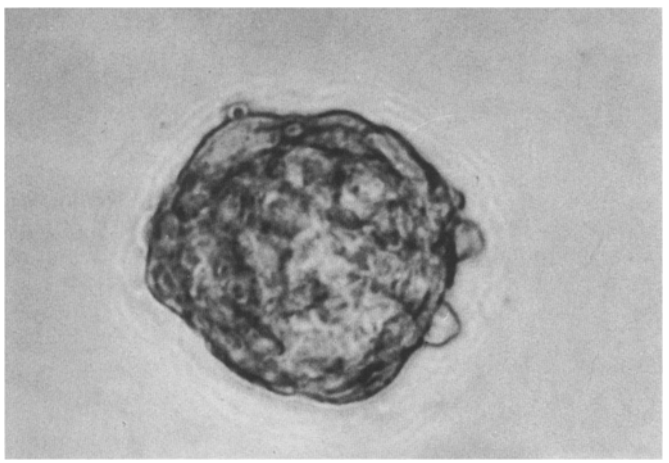

Fig. 3. A colony of chondrocytes formed from a single cell after 21 days of agarose-stabilized suspension culture

Table 1. Action of vitamin D metabolites on cell proliferation in logarithmic growth phase (day 13) of freshly isolated chondrocytes in monolayer culture[a]

Hormone added	% Cells of control[b]
Solvent control (0.05% ethanol)	100.0 ± 8.3
$1,25(OH)_2D_3$ 10^{-13} M	143.4 ± 16.5*
$1,25(OH)_2D_3$ 10^{-12} M	240.5 ± 16.1*
$1,25(OH)_2D_3$ 10^{-11} M	106.3 ± 4.2
$1,25(OH)_2D_3$ 10^{-10} M	87.5 ± 10.1
$1,25(OH)_2D_3$ 10^{-9} M	77.5 ± 16.3*
$1,25(OH)_2D_3$ 10^{-8} M	67.5 ± 14.1*
$25(OH)D_3$ 10^{-8} M	76.9 ± 4.5**
$24,25(OH)_2D_3$ 10^{-8} M	91.7 ± 12.5

* P <0.01 vs control;
** P <0.05 vs control $1,25(OH)_2D_3$, Iα, 25-Dihydroxyvitamin D_3; $25(OH)D_3$, 25-hydroxyvitamin D_3; $24,25(OH)_2D_3$, 24(R), 25-dihydroxyvitamin D_3
[a] Data represent the mean of six dishes ±NSD
[b] Cells were counted on day 13 in logarithmic growth phase

Saturation analysis of cultured chondrocytes (Fig. 2) shows specific binding of [^{3}H]-$1,25(OH)_2D_3$ approaching saturation at 3 nM $1,25(OH)_2D_3$. Apparent affinity (K_d) as determined from the linear regression curve on Scatchard analysis was 10^{-11} M (Fig. 1b). The maximal binding of $1,25(OH)_2D_3$ was dependent on the growth phase of the chondrocytes and was increased threefold in proliferating cells (12,780 bound molecules/cell versus 4,538 molecules/cell in conrols, Fig. 2). Similar increase in [^{3}H]-$1,25(OH)_2D_3$ binding was obtained whether cells were initially seeded at low or higher densities, with the growth arrest occurring after 10 days of culture, or were analysed on day 10 in logarithmic growth phase and day 20 (plateau phase).

Effects of $1,25(OH)_2D_3$ on proliferation of growth plate chondrocytes

To investigate whether $1,25(OH)_2D_3$ or other vitamin D metabolites show effects on chondrocyte growth, cell number, [^{3}H]-thymidine incorporation and colony formation were monitored. $1,25(OH)_2D_3$ added at 10^{-12} M to the medium containing delipidated FCS, stimulated proliferation significantly compared with controls. In contrast, at concentrations of 10^{-9} M and above, cell proliferation was reduced (Table 1). To exclude toxicity resulting from charcoal treatment of FCS, batches subjected to one or three repetitive charcoal treatments were compared; cell proliferation and viability were not significantly different (data not shown).

Rat tibia epiphyseal chondrocytes cultured in 0.5% agarose-stabilized suspension cultures formed small colonies after a culture period of 7–10 days. After 21 days the colonies were easily detected under the microscope (Fig. 3). Colonies consisted of varying numbers of chondrocytes and were embedded in a matrix, which was stained by alcian blue. When cultures were grown in delipidated FCS, $1,25(OH)_2D_3$ had a significant stimulatory

Table 2. Effects of $1,25(OH)_2D_3$ on colony formation of chondrocytes in agarose suspension culture[a]

Hormone added	% Colonies of control	Cloning efficiency
Solvent control (0.05% ethanol)	100.0 ± 11.7	1.14 ± 0.24
$1,25(OH)_2D_3$ 10^{-12} M	164.2 ± 21.6*	1.87 ± 0.40*
$1,25(OH)_2D_3$ 10^{-10} M	87.9 ± 20.3	0.99 ± 0.20
$1,25(OH)_2D_3$ 10^{-8} M	8.20 ± 22.6	0.94 ± 0.20
$1,25(OH)_2D_3$ 10^{-6} M	73.8 ± 19.4	0.84 ± 0.16

* P <0.03 vs control

[a] After 21 days the cultures were terminated by fixation in buffered formaldehyde (4%). The colonies were counted in 100 squares (2-mm grid) with an inverted light microscope. Cloning efficiency was calculated from colonies formed/1,000 seeded cells. Values represent the mean of six dishes ±NSD (2 experiments)

Table 3. Action and interaction of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ on cell proliferation in the logarithmic growth phase (day 10) of freshly isolated chondrocytes in monolayer culture[a]

Hormones added	% Cells of control
Solvent control (0.05% ethanol)	100.0 ± 6.5
10^{-12} M $24,25(OH)_2D_3$	107.8 ± 6.6
10^{-8} M $24,25(OH)_2D_3$	103.7 ± 12.2
10^{-12} M $1,25(OH)_2D_3$	152.4 ± 11.0*
10^{-12} M $1,25(OH)_2D_3$+10^{-8} M $24,25(OH)_2D_3$	164.2 ± 5.9*
10^{-12} M $1,25(OH)_2D_3$+10^{-12} M $24,25(OH)_2D_3$	156.6 ± 9.1*
10^{-8} M $1,25(OH)_2D_3$	73.5 ± 12.2*
10^{-8} M $1,25(OH)_2D_3$+10^{-8} M $24,25(OH)_2D_3$	75.1 ± 15.1*
10^{-8} M $1,25(OH)_2D_3$+10^{-12} M $24,25(OH)_2D_3$	76.9 ± 16.3*

* P <0.05 vs control

[a] The data represent the mean ±NSD of six dishes

effect at 10^{-12} M and a slight inhibitory effect which closely missed statistical significance at 10^{-10} M or above (Table 2). The increased number of colonies formed was due to increased cloning efficiency (1.14 ± 0.24 and 1.87 ± 0.40/1,000 seeded cells for solvent control and 10^{-12} M $1,25(OH)_2D_3$ respectively).

To prove whether this biphasic effect is specific for $1,25(OH)_2D_3$, the effect of $24,25(OH)_2D_3$ on cell proliferation, administered either alone or together with $1,25(OH)_2D_3$, was investigated in monolayer cultures. $24,25(OH)_2D_3$, which showed no specific binding to chondrocytes (G. Klaus, unpublished work), had no efect on proliferation, whether administered at 10^{-12} M or 10^{-8} M. When $24,25(OH)_2D_3$ was administered at 10^{-12} M or 10^{-8} M with $1,25(OH)_2D_3$ (10^{-12} M or 10^{-8} M), the action of $1,25(OH)_2D_3$ on proliferation was not modified (Table 3).

Immunohistochemical distribution of the $1,25(OH)_2D_3$ receptor in vivo by growth plate chondrocytes

Distribution of the receptor in chondrocytes of the growth plate was studied by examining tibial growth plates of rabbits ex vivo. As demonstrated in Fig. 4, the nuclei of the chondrocytes reacted with anti-receptor mAb (mAb 9A7τ2b) diluted 1 : 1,000. Staining intensity was highest in proliferating and hypertrophic chondrocytes (Fig. 4A, B). Specificity of the staining reaction was confirmed using rat mAb 9A7T2b or with similar concentrations of IgG of the same subclass (mAb directed against unrelated antigen, Fig. 4C).

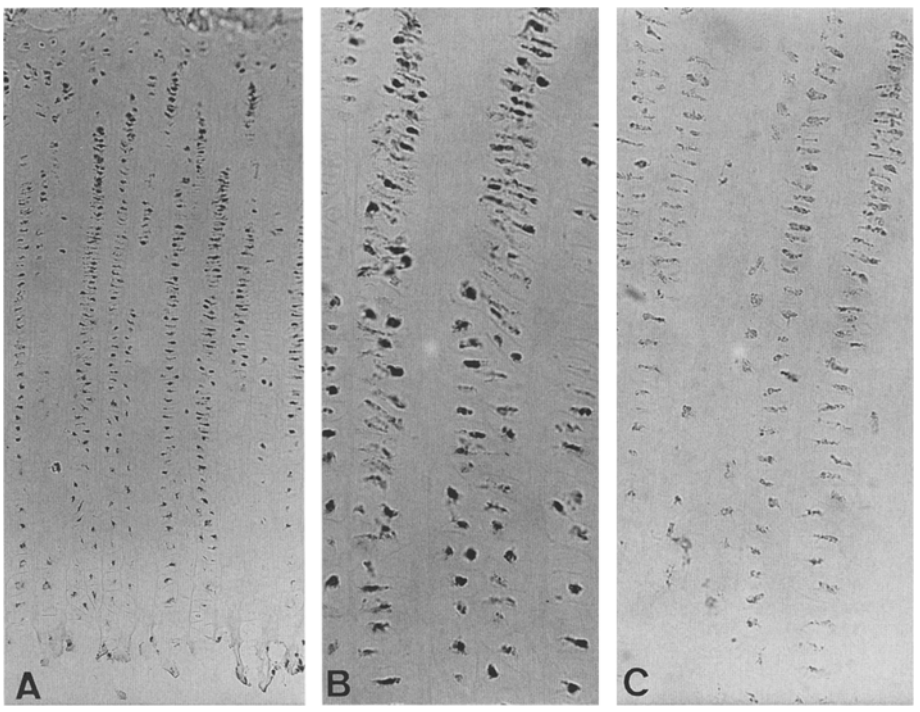

Fig. 4A–C. $1,25(OH)_2$-vitamin D_3 receptor expression in rabbit tibial growth plates. **A** Cryostat sections (5 μm) of rabbit tibial growth plates were incubated with monoclonal antibody (mAb) 9A7τ2b 1 : 1,000 and stained with the labelled avidin-biotin method. Note strong staining of chondrocyte nuclei, most intense in the proliferative and early hypertrophic zone (×160). **B** Higher magnification of proliferative zone stained with mAb 9A7τ2b. **C** Negative control showing no staining when the section was incubated with rat IgG instead of mAb 9A7τ2b (×360)

Discussion

$1,25(OH)_2D_3$ receptors have been demonstrated in non-mammalian embryonic chondrocytes [3]. The present study documents that receptors are also present in growth plate chondrocytes of mammals in vitro and ex vivo (Figs. 1, 2, 4). Specific binding of $1,25(OH)_2D_3$ was consistently demonstrable in growth cartilage of proximal tibia and in primary cultures of growth cartilage cells, but [within the sensitivity of the method (5 fmol/mg protein)] not in xiphoid cartilage. K_d, the sedimentation coefficient and the relative binding of other vitamin D metabolites (Figs. 1, 2) corresponded to the data available for the classical vitamin D receptor found in other tissues [12, 13, 16–18].

Expression of the receptor was dependent on the growth phase and cell maturation. The receptor was up-regulated in rapidly proliferating cells, i.e. in the phase of logarithmic growth in vitro. Similar results were obtained in this laboratory with endothelial cells [12]. In these experiments, evidence was also provided that receptor expression is modulated by protein C kinase-dependent mechanisms.

The regulation of vitamin D receptor numbers may be important in the modulation of the $1,25(OH)_2D_3$ response [3, 19]. $1,25(OH)_2D_3$ inhibits proliferation of several cell lines [18, 20] and biphasic effects on growth have been demonstrated in the human breast cancer cell line T47D [17], human bone cells [21] and fibroblasts [22].

To remove vitamin D metabolites present in FCS, this was delipidated by charcoal stripping; afterwards no $1,25(OH)_2D_3$ was demonstrable. Charcoal treatment of FCS lowered proliferation of the cells, but had no toxic effects. Our study clearly demonstrates tht $1,25(OH)_2D_3$ stimulates proliferation of growth plate chondroxytes at low concentration (10^{-12} M) and causes inhibition at concentrations of 10^{-10} M and above. The effect of $1,25(OH)_2D_3$ on cell proliferation was specific, because no effect was seen with dexamethasone (data not shown) or $24,25(OH)_2D_3$, whether administered alone or with $1,25(OH)_2D_3$. A proliferative effect was only obtained after FCS was delipidated by charcoal treatment.

The concentration of $1,25(OH)_2D_3$ (10^{-12} M) causing stimulation of chondrocytes in this study is lower than the total concentration of $1,25(OH)_2D_3$ in circulating blood. However, the physiological concentrations of $1,25(OH)_2D_3$ present in the growth plate have not been investigated and might be considerably lower than those found in serum.

It is well documented that primary isolates of chondrocytes grown in monolayer cultures for extended periods of time lose the expression of their differentiated properties, often referred to as dedifferentiation [23]. Chondrocytes grown in agarose-stabilized suspension cultures retain their differentiated phenotype [10]. Therefore, it is important to confirm results obtained from monolayer cultures in stabilized suspension cultures. In fact, both the low-dose stimulatory and higher-dose inhibitory effects of $1,25(OH)_2D_3$ were observed in agarose-stabilized suspension cultures.

The biphasic effect of $1,25(OH)_2D_3$ on proliferation of rat tibial growth plate chondroxytes is in line with the results of Takigawa et al. [4], who noted a stimulatory effect at 10^{-8} M $1,25(OH)_2D_3$ on proliferation of rabbit costal chondrocytes. The differences in effective $1,25(OH)_2D_3$ concentrations are difficult to explain, but may be due to different culture techniques, cell origin, loss or absorption of $1,25(OH)_2D_3$.

The $1,25(OH)_2D_3$ receptor is expressed throughout the growth plate, but receptor density per cell seems to be highest in proliferating and early hypertrophic chondrocytes, as demonstrated by the most intensive staining in these regions by immunohistochemistry. For immunohistochemical staining rabbits had to be used, because the mAb 9A7τ2b had been obtained from rodents [24]. During our studies Iwamoto et al. [25] reported that the highest specific binding of $1,25(OH)_2D_3$ throughout the growth plate is present in slices of hypertrophic chondrocytes, obtained from rat rib growth plate.

A number of studies have indicated a specific role for $24,25(OH)_2D_3$ in the function of growth plate chondrocytes [6, 7, 9, 26, 27]. We did not observe any effect of $24,25(OH)_2D_3$ on cell proliferation or binding of $24,25(OH)_2D_3$ to a specific binding molecule. Furthermore, $24,25(OH)_2D_3$ did not modify the actions of $1,25(OH)_2D_3$. In summary, the present study indicates that $1,25(OH)_2D_3$, but not $24,25(OH)_2D_3$ is an important factor for the proliferation of growth plate chondrocytes in rats.

Acknowledgements. This work was supported by a grant from the Deutsche Forschungsgemeinschaft KL 320/1. We would like to thank Professor O. G. Isaksson (Department of Physiology, University of Gothenburg, Sweden) for his help in establishing the cell cultures.

References

1. Portale AA, Booth BE, Tsai HC, Morris RC Jr (1982) Reduced plasma concentration of 1,25-dihydroxyvitamin D in children with moderate renal insufficiency. Kidney Int 21: 627–632
2. Chesney RW, Mehls O, Anast CS (1986) Renal osteodystrophy in children: the role of vitamin D, phosphorus and parathyroid hormone. Am J Kidney Dis 7: 275–284
3. Suda S, Takahashi N, Shinki T, Yamaguchi A, Yoshiki S, Suda T (1985) 1α,25-dihydroxyvitamin D_3 receptors and their action in embryonic chick chondrocytes. Calcif Tissue Int 37: 82–90
4. Takigawa M, Enomoto M, Shiral E, Nishii Y, Suzuki F (1988) Differential effects of 1α,25-dihydroxycholecalciferol and 24R,25-dihydroxycholecalciferol on the proliferation and the differentiated phenotype of rabbit costal chondrocytes in culture. Endocrinology 122: 831–839
5. Silbermann M, Von Der Mark K, Mirsky N, Von Menxel M, Lewinson D (1987) Effects of increased doses of 1,25-dihydroxyvitamin D_3 on matrix and DNA synthesis in condylar cartilage of suckling mice. Calcif Tissue Int 41: 95–104
6. Schwartz Z, Boyan B (1988) The effects of vitamin D metabolites on phospholipase A2 activity of growth zone and resting zone cartilage cells in vitro. Endocrinology 122: 2191–2197
7. Harmand MF, Thomasset M, Rouais F, Ducassou D (1984) In vitro stimulation of articular chondrocyte differentiated function by 1,25-dihydroxycholecalciferol or 24R,25-dihydroxycholecalciferol. J Cell Physiol 119: 359–365
8. Silbermann M, Mirsky N, Levitan S, Weisman Y (1983) The effect of 1,25-dihydroxyvitamin D_3 on cartilage growth in neonatal mice. Metab Bone Dis Relat Res 4: 337–345
9. Soemjen D, Earon Y, Harell S, Shimshoni Z, Weisman Y, Harell A, Kaye AM, Binderman I (1987) Developmental changes in responsiveness to vitamin D metabolites. J Steroid Biochem 27: 807–813

10. Benya PD, Shaffer JD (1982) Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. Cell 30: 215–224
11. Lindahl A, Isgaard J, Carlsson L, Isaksson OGP (1987) Differential effects of growth hormone and insulin-like growth factor 1 on colony formation of epiphyseal chondrocytes in suspension culture in rats of different ages. Endocrinology 121: 1061–1069
12. Merke J, Milde P, Lewicka S, Hügel U, Klaus G, Mangelsdorf DJ, Haussler MR, Rauterberg EW, Ritz E (1989) Identification and regulation of 1,25-dihydroxyvitamin D_3 receptor activity and biosynthesis of 1,25-dihydroxyvitamin D_3. Studies in cultured bovine aortic endothelial cells and human dermal capillaries. J Clin Invest 83: 1903–1915
13. Merke J, Klaus G, Hügel U, Waldherr R, Ritz E (1986) No 1,25-dihydroxyvitamin D receptors on osteoclasts of calcium-deficient chicken despite demonstrable receptors on circulating monocytes. J Clin Invest 77: 312–314
14. Wecksler WR, Norman AW (1979) An hydroxylapatite batch assay for the quantitation of 1,25-dihydroxyvitamin D_3 receptor complexes. Anal Biochem 92: 314–323
15. Scatchard G (1949) The attraction of proteins for small molecules and ions. Ann NY Acad Sci 51: 660–671
16. Chen TI, Feldman D (1981) Regulation of $1,25(OH)_2D_3$ receptors in cultured mouse bone cells. Correlation of receptor concentration with the rate of cell division. J Biol Chem 256: 5561–5566
17. Freake HL, Marcocci C, Iwasaki J, MacIntyre I (1981) 1,25-dihydroxyvitamin D_3 specifically binds to a human breast cancer cell line (T47D) and stimulates growth. Biochem Biophys Res Commun 101: 1131–1138
18. Merke J, Schwittay D, Fürstenberger G, Gross M, Marks F, Ritz E (1985) Demonstration and characterisation of $1,25(OH)_2D_3$ receptors in basal cells of neonatal and adult mice. Calcif Tissue Int 37: 257–267
19. Chen TL, Huschka PV, Cabrales S, Feldman D (1986) The effects of 1,25-dihydroxyvitamin D_3 and dexamethasone on rat osteoblast-like primary cell cultures: receptor occupancy and functional expression patterns for three different bioresponses. Science 235: 250–259
20. Suda T, Abe E, Miyaura C, Tanaka H, Shiina Y, Hayashi T, Nagasawa H, Chida K, Hashiba H, Fukushima M, Nishii Y, Kuroki T (1985) Modulation of cell differentiation and tumor promotion by 1,25-dihydroxyvitamin D_3. In: Norman AW, Schaefer K, Grigoleit H-G, Herrath DV (eds) Vitamin D: chemical, biochemical and clinical update. Gruyter, Berlin, pp 187–196
21. Skjodt H, Gallagher JA, Beresford JN, Couch M, Poser JW, Russel RGG (1984) Vitamin D metabolites regulate osteocalcin synthesis and proliferation of human bone cells in vitro. J Endocrinol 105: 391–396
22. Barsonay J, McKoy W, DeGrange DA, Liberman UA, Marx SJ (1989) Selective expression of a normal action of 1,25-dihydroxyvitamin D receptor in human skin fibroblasts with hereditary severe defects in multiple actions of that receptor. J Clin Invest 83: 2039–2101
23. Von Der Mark K, Gauss V, Von Der Mark H, Müller P (1977) Relationship between cell shape and type of collagen synthesized as chondrocytes lose their phenotype in culture. Nature 267: 531–532
24. Pike JW, Marion SL, Donaldson CA, Haussler MR (1983) Serum and monoclonal antibodies against the chick intestinal receptor for 1,25-dihydroxyvitamin D_3. J Biol Chem 258: 1289–1296
25. Iwamoto M, Sato K, Nakashima K, Shimazu A, Kato Y (1989) Hypertrophy and calcification of rabbit permanent chondrocytes in pelleted cultures: synthesis of alkaline phosphatase and 1,25-dihydroxycholecalciferol receptor. Dev Biol 136: 500–507
26. Binderman I, Soemjen D (1984) 24,25-dihydroxycholecalciferol induces growth of chick cartilage in vitro. Endocrinology 115: 430–432
27. Corvol M, Uimann U, Garabedian M (1980) Specific nuclear uptake of 24,25-dihydroxycholecalciferol, a vitamin D metabolite biologically active in cartilage. FEBS Lett 116: 273–276